REMARKS

Claim Rejections - 35 USC § 112, first paragraph

Claims 14-21 and 27 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement in that the claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention without undue experimentation.

As stated by the examiner, the instant claims are drawn to methods for identification of T-cell stimulating protein fragments comprising the following steps:

detecting an amino acid sequence of an antigen;

subdividing the amino acid sequence into fragments;

synthesizing at least one protein fragment;

incubating a suspension containing T-cells with the protein fragment;

identifying an induced T-cell cytokine or activation of a marker by flow cytometry;

assigning experimental runs in which T-cells have been stimulated and the stimulation has been recognized by a T-cell cytokine or an activation marker.

This method requires that the incubation time of the protein fragment(s) with cell suspension containing T cells be of a duration "sufficiently long so that the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules said taking up being sufficient when an unambiguous identification of stimulated T cells is possible" and "... sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T cells do not occur".

The examiner states that the specification is silent with regard to what incubation time for a given protein fragment and with regard to how one would determine if "particular T cells" have proliferated and elimination has occurred. Given that immunological (T cell) responses are antigen dependent, the examiner argues that one would not be able to predict what amount of

time would be sufficient for the protein fragment or fragments to be sufficiently taken up by the major histocompatibility antigen (MHC) molecules said taking up being sufficient when an unambiguous identification of stimulated T cells is possible and sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T cells do not occur.

The examiner concludes by stating that the specification provides no working examples that provide guidance in making such a prediction. Consequently, since the amount of experimentation required to determine if a given protein or protein fragment would the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules said taking up being sufficient when an unambiguous identification of stimulated T cells is possible and sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T cells do not occur would be undue, the specification is not enabling for the claimed method.

Applicant respectfully disagrees.

Applicants respectfully submit that anyone skilled in the art, i.e. in the setting up cell culture experiments, is aware that proliferation typically occurs not before 24 hours of stimulating T-cells.

Since T-cells recognize T-cell epitopes (MHC-molecule plus a bound peptide) as a specific spatial structure (including electric charges), the time to proliferation following stimulation does not depend on the nature of the protein fragment. Even if protein fragments require cleavage before they can bind, such cleavage occurs rapidly (see *Sherman et al.*, 3 Exp Med, 1992, 175(5):1221-1226, copy attached).

Also, protein fragments such as described in the application do not differ significantly *in* regards of the time required to be taken up by the MHC.

In particular, T-cell Blastogenesis *is known to anyone* skilled *in the art as the* process preceding proliferation, and occurs no sooner than 24 hours following T-cell activation.

This is known to anyone skilled in the art, and can be found in standard immu*nology* textbooks, *such as Abbas,* Abdul K., Cellular and Molecular Immunology, Saunders

Company, 1997, ISBN 0-7216-4024-9 (copy attached). On page 160, Figure 7 - 9, the time course of events in T-cell activation is depicted, It is shown that DNA-Synthesis begins approximately 24 hours after T-cell activation. *Wells et al.* 1997, The Journal of Clinical Investigation, 100(12); 3173-3183 (copy attached), show that 24 hours after in vitro activation of T-cells no cell divisions are detectable, whereas such divisions are seen at 48 hours (page 3175, Figure 1). Numerous examples can be quoted showing that T-cell proliferation is not to be expected until about 24 to 48 hours after activation. This is the time required for cytokine gene transcription, cytokine synthesis and secretion, and eventually DNA-synthesis (see *Abbas*, above, for more details).

A time sufficiently short to avoid proliferation and sufficiently long to allow pep-tide uptake into the MHC-binding groove would therefore be understood as a time between several minutes to approximately 24 hours after stimulation. The exact time until proliferation will depend on the type of T-cells and the conditions and *cannot be* precisely predicted *for every possible system.*

The literature available prior to the filing date gave sufficient guidance as to the time frame in which proliferation can be expected to occur, this was described in most if not all immunology textbook as one of the basic facts of T-cell activation, It was basic knowledge to anyone skilled in the art. The application clearly specifies that this time can be 6 hours. As a result, the examiners conclusion that no guidance is given as to the length of the required time is not understood.

Typically peptide loading of antigen presenting cells is rapid and occurs certainly within 30 to 45 minutes, even if peptides need to be clipped/shortened. For example, Sherman et al. demonstrate that lysis of target cells by specific effector cells occurs within 20 minutes of adding the cognate peptide (Figure 2, page 1223). The presentation of the peptide depended on prior cleavage by proteolytic serum activity, however, the time to effective presentation was only about 15 minutes.

Thus, applicant believes that one skilled in the art is readily able to utilize the present disclosure in conjunction with the existing art to determine the appropriate time frame.

***Claim Rejections - 35 USC § 112*, second paragraph**

Claims 14-21 and 27 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for

failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention in that claims 14 and 27 contain the phrase "selection and proliferation accompanied by the specific elimination of particular T cells do not occur". The examiners position is that it is unclear what is meant by said term "particular T cells" and by the term "specific elimination".

Applicant has deleted the objectionable words "particular" and "specific" thus obviating this ground for rejection.

Claim 16 stands rejected under 35 U.S.C. 112, second paragraph, as vague and indefinite in the use of the phrase "the protein fragment essentially in a state bound to MHC class I or class II molecules.

Applicant respectfully traverses this ground for rejection but solely for the sake of clarity, applicants have amended claim 16.

Not all peptides will bind and the successful binding to the MHC is one important step in the antigen-presentation process, i.e. is a prerequisite for the T cell response. Only peptides that are good binders will be selected and deduce T cell responses, which is the principle of the method of the present application.

Conclusion

Applicant believes these remarks and the claim amendments are sufficient to obviate the grounds for rejection presented in the outstanding office action and respectfully requests allowance of the pending claims. Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,
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